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Characterization, HPLC method development and impurity identification for 3,4,3-LI(1,2-HOPO), a potent actinide chelator for radionuclide decorporation

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ABSTRACT

3,4,3-LI(1,2-HOPO), 1,5,10,14-tetra(1-hydroxy-2-pyridon-6-oyl)-1,5,10,14-tetraazatetradecane, is a potent octadentate chelator of actinides. It is being developed as a decorporation treatment for internal contamination with radionuclides. Conventional HPLC methods exhibited speciation peaks and bridging, likely attributable to the agent's complexation with residual metallic ions in the HPLC system. Derivatization of the target ligand in situ with Fe(III) chloride, however, provided a single homogeneous iron-complex that can readily be detected and analyzed by HPLC. The HPLC method used an Agilent Eclipse XDB-C18 column (150 mm × 4.6 mm, 5 μm) at 25 °C with UV detection at 280 nm. A gradient elution, with acetonitrile (11% to 100%)/buffer mobile phase, was developed for impurity profiling. The buffer consisted of 0.02% formic acid and 10 mM ammonium formate at pH 4.6. An Agilent 1200 LC-6530 Q-TOF/MS system was employed to characterize the [Fe(III)-3,4,3-LI(1,2-HOPO)] derivative and impurities. The proposed HPLC method was validated for specificity, linearity (concentration range 0.13–0.35 mg/mL, $r = 0.9999$), accuracy (recovery 98.3–103.3%), precision ($RSD \leq 1.6\%$) and sensitivity (LOD 0.08 μg/mL). The LC/HRMS revealed that the derivative was a complex consisting of one 3,4,3-LI(1,2-HOPO) molecule, one hydroxide ligand, and two iron atoms. Impurities were also identified with LC/HRMS. The validated HPLC method was used in shelf-life evaluation studies which showed that the API remained unchanged for one year at 25 °C/60% RH.

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1. Introduction

3,4,3-LI(1,2-HOPO), 1,5,10,14-tetra(1-hydroxy-2-pyridon-6-oyl)-1,5,10,14-tetraazatetradecane (Fig. 1), abbreviated herein as HOPO, is a potent octadentate chelator of actinides. It is being developed as a decorporation agent for internal contamination with radionuclides [1].

All actinides are radioactive and, when internalized, can damage and induce cancer in bone, liver, and lungs if inhaled [2–5]. Decontamination of exposed persons is needed to reduce the consequences of the radionuclide intake. The accepted way to reduce the health risks of internally deposited actinides is to accelerate

their excretion with chelating agents, and diethylenetriaminepentaacetic acid (DTPA) is used clinically for that purpose. Actinides are known to penetrate biological iron transport and storage systems indicating that actinide ions will likely form stable complexes with the Fe(III)-binding units found in selective natural iron chelators (siderophores). A biomimetic approach demonstrated *in vivo* Pu(IV) chelation of synthetic multidentate ligands that were based on the backbone structures and Fe(III)-binding groups of bacterial siderophores [6]. New actinide chelators, including the octadentate 3,4,3-LI(1,2-HOPO) and the tetradentate 5-LIO(Me-3,2-HOPO), are effective to decorporate Pu(IV), Am(III), U(VI), and Np(IV,V).

Chemical analysis showed high affinity of HOPO to Ce(IV), Th(IV), U(IV) and predictable high affinity to Np(IV) and Pu(IV) [7,8]. These analytical results corroborate the *in vivo* chelation efficacy of HOPO and validated their selection for further development as therapeutic actinide decorporation agents. In animal models, both

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Fig. 1. Structure of 3,4,3-LI(1,2-HOPO). 1,5,10,14-tetra(1-hydroxy-2-pyridon-6-yl)-1,5,10,14-tetraazatetradecane.

3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) showed oral activity and acceptable toxicity profiles at effective dose levels[1]. An update on the preclinical development of the two new ligands is given by Rebecca Abergel et al. in 2010, describing the synthesis scale-up, analytical methods, in vivo actinide removal efficacy, safety and toxicity studies and cellular-level toxicity studies[9]. More studies testing different variables further support the efficacy and safety of the two compounds 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) [10,11].

As part of the pre-clinical program sponsored by NIH-RAID, physico-chemical characterization, HPLC method development/validation and shelf-life evaluation have been undertaken. Conventional HPLC methods exhibited speciation peaks and bridging, likely attributable to complexation with residual metallic ions in the eluent. As the compound was originally designed to be a plutonium (IV) scavenger based on the similar biochemical properties of plutonium (IV) and iron (III), we took advantage of these characteristics and successfully used iron (III) ions to promote chelate formation with HOPO, to the exclusion of other metals with lesser affinity. The compound is converted solely to its iron complex by reaction with ferric chloride and analyzed by HPLC using an Eclipse XDB C18 column and gradient elution with acetonitrile and ammonium formate buffer. A stability indicating HPLC method was developed and validated in accordance with ICH guideline Q2(R1). Impurities were identified with LC-MS/MS with accurate mass data.

2. Material and methods

2.1. Chemicals and reagents

3,4,3-LI(1,2-HOPO) (NSC 749716) was provided by the National Cancer Institute (Bethesda, MD, USA). HPLC grade acetonitrile (ACN) and hydrogen peroxide (H_2O_2) 30% solution were purchased from Mallinckrodt (Paris, KY, USA). Water was purified through a Millipore Super-Q Pure Water System (Waltham, MA, USA). Solutions of hydrochloric acid (HCl) and sodium hydroxide (NaOH) were prepared from Dilute-it Analytical Concentrate (J.T. Baker, Phillipsburg, NJ, USA). Formic acid, ammonium formate, anhydrous iron (III) chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. HPLC

An Agilent 1100 HPLC system (Wilmington, DE, USA) equipped with a solvent degasser, pump, autosampler, and PDA detector was used in the study. Agilent ChemStation for LC (A 10.02) software was used for instrument operation control and data collection. A Phenomenex Luna C18(2) column (5 μ m, 150 $\times$ 4.6 mm I.D.; Torrance, CA, USA) was initially used during method development, then it was changed to an Agilent Eclipse XDB-C18 column (5 μ m, 150 $\times$ 4.6 mm I.D.; Wilmington, DE, USA) for optimal peak shape.

The Eclipse C18 column was held at 25 °C. The mobile phase was a combination of solvent A (acetonitrile/water, 5:95, v/v, containing 0.02% formic acid and 10 mM ammonium formate) and solvent B (acetonitrile). The gradient program was: 0–5 min, 6% solvent B and 94% solvent A; 5–30 min, linear gradient to 28% solvent B and 72% solvent A; 30–40 min, linear gradient to 100% solvent B and 0% solvent A; 40–50 min, re-equilibrate at 6% solvent B and 94% solvent A before the next injection. The injection volume was 20 μ L. The elution flow rate was 1.0 mL/min, and the detection wavelength was set at 280 nm.

LC-MS was performed on an Agilent LC/MS system consisting of an Agilent 1200 binary LC pump, a temperature-controlled autosampler, a PDA UV detector, and a 6530 Accurate Mass Q-TOF mass spectrometer (Wilmington, DE, USA). The mass spectrometer was equipped with a JetStream® ESI probe operating at atmospheric pressure. The ESI source parameter settings were: mass range m/z 100–1000, gas temperature 350 °C, gas flow 10 L/min, nebulizer 50 psi, sheath gas temperature 400 °C, sheath gas flow 12 L/min, capillary voltage (Vcap) 3500 V, nozzle voltage 500 V, fragmentor 200 V, skimmer 65 V, octopole RF (OCT 1 RF Vpp) 750 V. Tandem mass spectrometry was performed using ramped collision energy at slope 3 and offset 10. The LC conditions used for identification of impurities and decomposition products of 3,4,3-LI(1,2-HOPO) were the same as those described above.

2.3. Environmental chamber for photo stressed decomposition

The forced degradation study under UV and visible light was carried out in the ES 2000 Environmental Chamber (Environmental Specialties, Inc., Raleigh, NC, USA), equipped with a cool white lamp (8.0 kilolux) and a UV-A lamp (14.00 W/m²), in conformance with the ICH Q1B option 2 for photostability testing. Temperature and humidity conditions were set at 25 °C/60% RH.

2.4. Sample preparation

Assay standards and samples are prepared at a concentration of 0.25 mg/mL dissolved in the diluent of 0.3 mg/mL $FeCl_3$ in acetonitrile/water (20:80, v/v) containing 0.25% formic acid. The standard or sample solutions are heated at 40 °C for 2 h. Formic acid is needed in the diluent to prevent hydrolysis of the iron (III) element.

Forced degradation was conducted under the degradation condition given in Table 1. After the stress treatment, the samples were

Table 1
Forced degradation condition.

Sample description	Degradation condition
Water	1.0 mg/mL in H_2O , 80 °C, 2.5 h
Acid	2.0 mg/mL in 1 N HCl, 80 °C, 60 min, neutralize with 1 N NaOH
Base	2.0 mg/mL in 0.1 N NaOH, 80 °C, 30 min, neutralize with 0.1 N HCl
Oxidation	1.0 mg/mL in 0.03% H_2O_2 , 80 °C, 60 min
Solid dry heat	Solid heated at 80 °C, 24 h
Solid photo	UV light for ~10 min, cool white light, 10 min
Solid photo (control)	UV light for ~10 min, cool white light, 10 min, wrapped in foil

Table 2
MS data for peaks in Fig. 2b.

Peak	m/z in positive mode
A	751 (HOPO + H^+)
B	804 (HOPO-2 H^+ + Fe^{3+})
C	775 (HOPO-2 H^+ + Al^{3+})
D (left side of the peak)	775 (HOPO-2 H^+ + Al^{3+})
E (top and right side of the peak)	804 (HOPO-2 H^+ + Fe^{3+})

Fig. 2. Chromatograms of 3,4,3-LI(1,2-HOPO). (a) Obtained from an Agilent 1100 LC-UV system. (b) Obtained from a Thermo Finnegan LC/MS system. (c) Obtained from a Thermo Finnegan LC/MS system after adding $\text{Fe}_2(\text{SO}_4)_3$ to the sample solution.

admixed with the diluent (0.3 mg/mL FeCl_3 in acetonitrile/water (20:80, v/v) containing 0.25% formic acid) to prepare solutions at a concentration of 0.25 mg/mL and then heated at 40 °C for 2 h before injection.

For validation of linearity, accuracy and precision, accurately weighed 0.25–0.75 mg portions of 3,4,3-LI(1,2-HOPO) were dissolved in 2.0 mL of the diluent to yield five solutions in the 0.125–0.375 mg/mL.

3. Results and discussion

3.1. HPLC method development

3.1.1. Problem encountered

The HPLC method development was explored with conventional reversed phase conditions on an ODS column using water and acetonitrile containing formic acid as the mobile phase. The

Fig. 3. Chromatograms of (a) the room temperature sample and (b) 40 °C heated 2 h sample.

unexpected appearance of multiple peaks with a purified sample was the major issue (Fig. 2a). The peak profile varied dramatically with the instrument system used (Fig. 2b). These observations indicate that the extraneous peaks may not represent impurities but are derivatives from the parent compound.

Since the compound is a strong metal chelator, the likely cause of multiple peaks may be due to complexes formed with free metals in the HPLC system. Initial efforts were made to purge the HPLC system by cleansing with concentrated nitric acid, adding EDTA to the mobile phase to scavenge free metallic ions, using PEEK column housing, and non-silica-based polymeric reversed phase columns. None of these measures was able to effectively eliminate the extraneous peaks.

3.1.2. Investigation of the multiple peaks by LC/MS

The nature of these peaks was first investigated with LC/MS. Fig. 2b is a chromatogram obtained from the LC/MS system showing a different profile from Fig. 2a, although the same HPLC column and mobile phase were used.

Table 2 provides the mass data and interpretation for peaks in Fig. 2b. The MS data showed that peak A has the intact molecular ion of m/z 751 for HOPO. Peaks B and E, with a molecular ion at m/z 804 represent two different forms of a monoiron (Fe^{3+}) complex, appearing as two peaks. Similarly, peaks C and D, having the same molecular ion of m/z 775, represent two distinct forms of a monoaluminum (Al^{3+}) complex.

3.1.3. Adding Iron (III) to eliminate multiple peaks

HPLC analysis of metal chelators has been challenging due to their ability to chelate with different metals leading to the formation of multiple related complexes. Several reports showed that the analysis of ethylenediaminetetraacetic acid (EDTA) could be accomplished by converting EDTA to a single metal complex, typically with iron (III) [12] and copper (II) [13–15]. An analogous approach was taken in the current HOPO analysis.

HOPO was designed to be a plutonium scavenger based on the similar biochemical properties of plutonium (IV) and iron (III). Since HOPO has high affinity for plutonium (IV), it was theorized that it also has a high affinity for iron (III). The above MS data also showed

Fig. 4. Accurate mass spectra of the main peak, peak X, and the proposed identities of these two peaks.

Fig. 5. HPLC chromatogram of 3,4,3-LI(1,2-HOPO), lot ML-11-276, 0.25 mg/mL in diluent.

that HOPO would readily chelate with iron (III). Based on these assumptions and observations, we attempted to use iron (III) as the preferred ion to chelate with HOPO so as to suppress or eliminate complex formation with any other metals by affinity competition and mass equilibrium.

Fig. 2c is a chromatogram obtained from the LC/MS system. By adding 0.5 mg/mL of $\text{Fe}_2(\text{SO}_4)_3$ to a 0.25 mg/mL HOPO solution, the multiple peaks disappeared and a single sharp peak was formed. MS indicated the molecular ion of this peak was m/z 875. With the help of accurate mass data obtained from a high resolution QTOF-MS, the HOPO-Fe complex was determined to be $[\text{HOPO}^4-\text{Fe}^{3+}_2\text{OH}]^+(m/z=875.1012)$.

3.1.4. Optimization

Since iron (III) can form a stable complex with HOPO and eliminate most of the extraneous peaks, it was selected for further

optimization. The goal was to develop a quantitative and stability indicating HPLC analysis. Therefore, the derivatization conditions had to be well controlled to yield reliable and consistent results.

Because commercial $\text{Fe}_2(\text{SO}_4)_3$ does not have specified water content while FeCl_3 has specified water content (either anhydrous or hexahydrate), for consistent results, anhydrous FeCl_3 was chosen to be the chelating reagent. The peak profiles were shown to be essentially identical with either $\text{Fe}_2(\text{SO}_4)_3$ or FeCl_3 reagents.

Comparison of the HOPO-Fe complex peak on a Phenomenex Luna C18 column and an Eclipse XDB-C18 column showed that the peak shape on the Eclipse column was near Gaussian-like (Tailing Factor 1.83) while the peak on the Luna column was fronting and tailing (Tailing Factor 0.89). Therefore, the Eclipse column was chosen for further optimization.

It was observed that the Fe/HOPO ratio and the diluent composition significantly affected the Fe-HOPO chelation reaction. There

Table 3

Proposed identities for impurities in 3,4,3-LI(1,2-HOPO), lot ML-11-276.

Rel. RT	Area (%)	Identity	Theoretical (m/z)	Measured (m/z)	Difference (ppm)	MS/MS fragment ions (m/z)
0.61	0.38 ± 0.03	B	667.1684	667.1681	0.45	650.1418
1.00	97.30 ± 0.06	NSC 749716	875.1017	875.1015	0.23	857.0903
1.16	0.13 ± 0.01	F	788.1847	788.1844	0.38	179.0818
1.21	0.56 ± 0.01	G	788.1847	788.1845	0.25	179.0813
1.42	0.17 ± 0.00	Q	725.1738	725.1731	0.97	693.1451, 650.1438
1.55	0.18 ± 0.01	H	822.1458	822.1454	0.49	213.0427
1.74	0.17 ± 0.01	I	822.1458	822.1453	0.61	213.0425
1.80	0.12 ± 0.01	C	894.2266	894.2258	0.89	285.1237, 195.0763
1.82	0.37 ± 0.00	R	915.1672	915.1628	4.81	306.0641
1.85	0.15 ± 0.01	J	894.2266	894.2251	1.68	285.1225, 195.0761
2.00	0.21 ± 0.02	S	840.1119	840.1107	1.43	No fragments observed
2.18	0.27 ± 0.01	T	840.1119	840.1097	2.62	231.0083

Fig. 6. Proposed structures of impurities >0.1% in 3,4,3-LI(1,2-HOPO), Lot ML-11-276.

seemed to be an optimal HOPO concentration and Fe:HOPO ratio that would allow the compound to be completely converted to a single complex (single peak) with acceptable solubility in the media (not precipitated). Diluent composition of ACN/H₂O also affected the complex formation and solubility. The Fe:HOPO complex had higher solubility in solvents containing higher organic portions. However, if the diluent contained more than 30% of ACN, it caused peak splitting due to solvent and mobile phase mismatch. Various Factors including HOPO concentration (0.25–0.5 mg/mL), FeCl₃ concentration (0.075–0.75 mg/mL) and ACN/H₂O ratio (0–50% ACN in water) were examined. The detailed test conditions and the results are provided in Supplementary Table 1.

The optimal HOPO concentration is ~0.25 mg/mL with 0.3 mg/mL FeCl₃ in ACN/H₂O (1:4). The molar ratio of HOPO to FeCl₃ was 1:5.5 at this concentration. The concentration (0.35 mg/mL) was set as the highest point of the linearity range, and the 0.25 mg/mL was set as the assay concentration. At this concentration, the sensitivity was sufficient to detect 0.1% impurity at 280 nm. In addition, 0.25% formic acid was included in the diluent as a hydrolytic preservative for FeCl₃.

It was observed that the Fe:HOPO chelation was a slow process, as the Fe:HOPO complex peak increased its area count with time. The peak area increased 1–3% within the first 4 h and gradually stabilized after 4 h. A minor form of the Fe-HOPO complex was also observed (Peak X in Fig. 3a). Time and temperature were

investigated to obtain an optimal condition for the Fe:HOPO complex formation. The results are given in Supplementary Table 2. It was found that heating the sample solution at 40 °C for 2 h accelerated the chelation process and converted the minor form (Peak X) to the major form of the complex (Fig. 3b).

LC-QTOF/MS was used to investigate the minor form of the Fe-HOPO complex. Fig. 4 shows the accurate mass spectra of the main peak and peak X, and the proposed structures of the two components. The measured masses of the two peaks are in good agreement (within 2 ppm) with the theoretically predicted mass from the proposed structures. The major form of the complex contains two Fe and one hydroxyl, while the minor form contains two Fe without hydroxyl group (exhibited as a doubly charged ion in MS).

The mobile phase buffer pH was critical to control the equilibrium of the two complex forms. In acidic mobile phase (0.05% formic acid), the minor form Peak X was more prominent. The optimal pH was 4.6 with a buffer containing 0.02% formic acid and 10 mM ammonium formate.

3.2. HPLC method validation

The optimized method, as presented in the method section, was validated in accordance with the current ICH guideline Q2 (R1). Fig. 5 is a typical HPLC assay chromatogram with the

Table 4

Stability results of 3,4,3-LI(1,2-HOPO), lot ML-11-203.

Test	Time 0	5 °C 12 months	25 °C/60% RH 12 months	40 °C/75% RH 6 months
Assay (as is)	96.0%	99.0%	95.3%	95.5%
Assay (anhydrous)	97.8%	101.9%	101.4%	98.5%
Chromatographic purity (main peak area %)	97.8%	98.7%	98.8%	98.8%
Water content	1.8%	2.9%	6.3%	3.0%

system suitability data presented on the figure. The optimized method was validated for specificity with forced degradation samples as described in the method section. Resolution (R_s) of the peaks preceding and following the active drug in all forced degradation samples were greater than 1.8. Linearity of the method was demonstrated by standard curve in the range of 0.125–0.375 mg/mL (50–150% of the target assay concentration). The sample peak area (A , mAU) versus drug concentration (C , mg/mL) was analyzed by linear least square regression which resulted in $A = 14589C + 176.63$ with an excellent correlation coefficient ($r = 0.9999$).

Accuracy and precision were established by evaluation recoveries and RSD values obtained with three test solution each at concentration of 0.125, 0.25 and 0.375 mg/mL corresponding to 50%, 100% and 150% of the target assay concentration. Recovery was calculated by comparing the theoretical concentration calculated from the calibration curve and the nominal concentration. The accuracy results showed recoveries between 98.5% and 103.3%. Precision was validated to be no greater than 1.86% RSD. Limit of detection (LOD) and limit of quantitation (LOQ) were shown to be 0.2 µg/mL and 0.5 µg/mL, respectively, using the criteria of $S/N > 3$ for LOD and $S/N > 10$ for LOQ. The sample solution stability was tested and shown to be stable at room temperature for 1 day, and at 5 °C for 3 days with less than 5% change.

3.3. Impurity characterization

Eleven impurity peaks with areas greater than 0.1% were detected in 3,4,3-LI(1,2-HOPO), lot ML-11-276 (Fig. 5 and Table 3). LC-QTOF/MS was used to identify these impurities. The observed molecular ion and major fragment ions are given in Table 3. Structures of the impurities were proposed based on accurate mass and MS/MS fragment interpretation (Fig. 6). The measured masses are in good agreement (within 5 ppm) with the theoretically predicted masses from the proposed structures (Table 3). MS/MS fragment interpretation (Supplementary Figure 1) and UV spectra (Supplementary Figure 2) for the Fe:HOPO complex and the observed impurities are provided in the supplementary data to further support the impurity characterization.

3.4. Stability study

The validated method was used in the stability study of the 3,4,3-LI(1,2-HOPO) drug substance. The assay, chromatographic purity and water content results are given in Table 4. Water contents were determined with Karl Fischer titration method. The assay results are reported without correction of water content (“as is” basis) as well as with water content correction (“anhydrous” basis). The stability results indicate that the compound is chemically stable at all tested conditions and time points.

4. Conclusion

An HPLC assay method has been developed and validated for 3,4,3-LI(1,2-HOPO). The method utilized Fe(III) chloride in situ derivatization to convert 3,4,3-LI(1,2-HOPO) to a single iron complex to the exclusion of other metals with lesser affinity, allowing the compound to be analyzed as one single peak. The HPLC

method separates 3,4,3-LI(1,2-HOPO) from its impurities and forced decomposition products. Identities of eleven impurities have been elucidated by MS spectral data. The assay method has been validated to be specific, linear ($r \geq 0.9999$), accurate (recovery 98.5–103.3%), precise ($RSD \leq 1.86\%$) and sensitive (LOD 0.2 µg/mL).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jpba.2014.10.015>.

References

- [1] P.W. Durbin, S. Lauriston, Taylor Lecture: the quest for therapeutic actinide chelators, *Health Phys.* 95 (2008) 465–492.
- [2] R.D. Evans, Radium poisoning: a review of present knowledge, *Am. J. Public Health Nation's Health* 23 (1933) 1017–1023.
- [3] R.D. Evans, Protection of radium dial workers and radiologists from injury by radium, *J. Ind. Hygiene Tox.* 25 (1943) 253–269.
- [4] R.D. Evans, R.S. Harris, J.W. Bunker, Radium metabolism in rats, and the production of osteosarcoma by experimental radium poisoning, *Am. J. Roentgenol. Radium Therap.* 52 (1944) 353–373.
- [5] N.B.o. Standards, Safe handling of luminous compounds, in: N.B.o. Standards (Ed.) NBS Handbook 27, National Bureau of Standards, Washington, DC, 1941.
- [6] A.E. Gorden, J. Xu, K.N. Raymond, P. Durbin, Rational design of sequestering agents for plutonium and other actinides, *Chem. Rev.* 103 (2003) 4207–4282.
- [7] G.J. Deblonde, M. Sturzbecher-Hoehne, R.J. Abergel, Solution thermodynamic stability of complexes formed with the octadentate hydroxypyridinonate ligand 3,4,3-LI(1,2-HOPO): a critical feature for efficient chelation of lanthanide(IV) and actinide(IV) ions, *Inorg. Chem.* 52 (2013) 8805–8811.
- [8] M. Sturzbecher-Hoehne, G.J.P. Deblonde, R.J. Abergel, Solution thermodynamic evaluation of hydroxypyridinonate chelators 3, 4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) for UO₂(VI) and Th(IV) decorporation, *Radiochim. Acta* 101 (2013) 359–366.
- [9] R.J. Abergel, P.W. Durbin, B. Kullgren, S.N. Ebbe, J. Xu, P.Y. Chang, D.I. Bunin, E.A. Blakely, K.A. Bjornstad, C.J. Rosen, D.K. Shuh, K.N. Raymond, Biomimetic actinide chelators: an update on the preclinical development of the orally active hydroxypyridinonate decorporation agents 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO), *Health Phys.* 99 (2010) 401–407.
- [10] E.E. Jarvis, D.D. An, B. Kullgren, R.J. Abergel, Significance of single variables in defining adequate animal models to assess the efficacy of new radionuclide decorporation agents: using the contamination dose as an example, *Drug Dev. Res.* 73 (2012) 281–289.
- [11] D.I. Bunin, P.Y. Chang, R.S. Doppalapudi, E.S. Riccio, D. An, E.E. Jarvis, B. Kullgren, R.J. Abergel, Dose-dependent efficacy and safety toxicology of hydroxypyridinonate actinide decorporation agents in rodents: towards a safe and effective human dosing regimen, *Radiat. Res.* 179 (2013) 171–182.
- [12] M. Sillanpaa, R. Kokkonen, M.L. Sihvonen, Determination of EDTA and DTPA as their Fe(III) complexes in pulp and paper mill process and waste waters by liquid chromatography, *Anal. Chim. Acta* 303 (1995) 187–192.
- [13] J. De Jong, A. Van Polanen, J.J.M. Driessen, Determination of ethylenediamine-tetraacetic acid and its salts in canned mushrooms by reversed-phase ion-pair liquid chromatography, *J. Chromatogr.* 553 (1991) 243–248.
- [14] L. Hall, L. Takahashi, Quantitative determination of disodium edetate in ophthalmic and contact lens care solutions by reversed-phase high-performance liquid chromatography, *J. Pharm. Sci.* 77 (1988) 247–250.
- [15] A.S. Kord, I. Tumanova, W.L. Matier, A novel HPLC method for determination of EDTA in a cataract inhibiting ophthalmic drug, *J. Pharm. Biomed. Anal.* 13 (1995) 575–580.